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VOLUME XLIX NUMBER 4

BOTANICAL GAZETTE

APRIL 1910

THE CELL STRUCTURE OF CLOSTERIUM EHRENBERGII AND CLOSTERIUM MONILIFERUM

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(WITH PLATES XVII AND XVIII)

Little has been contributed to our knowledge of the chromatophore and pyrenoids of Closterium since the time of NÄGELI and DEBARY, though several investigators have worked on the cell division, conjugation, and peculiar mode of locomotion of these plants.

NÄGELI (5) described the chromatophore, nucleus, end vacuoles, pyrenoids, and cell wall of the genus. He figures only *C. moniliferum* and *C. parvulum*, but mentions eleven other species as belonging to the genus, not including, however, *C. Ehrenbergii* among them. The chromatophore as described by him consists of three or more chlorophyll plates whose inner edges rest in the axis of the cell and whose outer edges extend to the cell wall. In optical cross section, obtained by examining the plant when it was standing on end, the chromatophore appeared to be made up of 3 to 15 plates extending radially from center to periphery. The nucleus he describes as a clear sac containing a denser body. In the colorless ends of the plant body are the clear vacuoles containing little black granules showing a motion which he calls molecular; these vacuoles are usually spherical, although at times irregular in shape. The pyrenoids, "little chlorophyll sacs," are arranged in *C. moniliferum* and *C. parvulum* in a single row of 2 to 20. The cell wall is striate. NÄGELI'S figures show, besides the external views, optical cross sections of *C. moniliferum* and *C. parvulum*. These are the only figures extant and they have been copied by OLTMANNS (17) and LOTSY (12).

DEBARY (5) also described Closterium, but adds little to NÄGELI'S account except that he recognizes the pyrenoids (*Amylonkerne*) as depositing starch in their interior, and made out that the little granules in the end vacuoles are rhombic plates with sharp angles, composed, as he discovered by tests, of gypsum. DEBARY used the term *Amylonkerne* for the pyrenoids because of the many analogies they offer to the cell nuclei. He had discovered by means of the iodin reaction that the outer layer is starch, and by means of the red coloration with sugar and sulfuric acid that the central body is composed of protein. He described the starch which appears in the outer layer as either forming a homogeneous shell on the outside of this central body, or in the cells rich in starch as composed of grains that give to the pyrenoid an irregularly cracked appearance. DEBARY was the first to give an adequate account of the appearance and chemical constitution of this body in Closterium, and little of real importance as to its chemical nature has been added since.

FISCHER (7) examined the crystals in the end vacuoles both as to their origin and composition. In his opinion they originate in the plasma, in which he claims there are many of them, from which they wander into the vacuoles.

LÜTKEMÜLLER (13) and a number of other observers have studied the structure and the beautiful and characteristic markings on the cell walls of the different species.

Later authors, working on other forms, have found little to correct in DEBARY'S account of the appearance and chemical composition of pyrenoids, but DEBARY gave no adequate suggestion as to their relation to starch formation. TIMBERLAKE (22) has reviewed the literature fairly thoroughly, and as little has appeared since his time all that will be necessary is a brief account of the points called in question in this paper.

According to SCHMITZ (18), who was the first to observe and figure the process, the pyrenoid reproduces by the simple method of pinching in two, the starch mass being divided between the two daughter pyrenoids and being replaced by growth on their sides where the division had occurred. SCHMITZ'S work, which was done on Hyalotheca, has been confirmed by CHMIELEVSKY (3) on Spirogyra, KLEBAHN (9) on Cosmarium, KARSTEN (8) on Grammatophora, STRASBURGER (20) on Zygnema, and TIMBERLAKE (21) on Hydrodictyon. In addition to reproduction by division, it is frequently assumed that they may arise *de novo* in the chromatophore, or at least arise from bodies too minute to be seen with the microscope. The apparent disappearance of the pyrenoids is characteristic of the time of spore formation and has been observed by STRASBURGER (**20**) in Cladophora, KLEBS (**11**) in Chlamydomonas and in Hydrodictyon, and OVERTON (**18**) in Volvox. They reappear when the spores begin to grow again and apparently arise *de novo.*

The commonly accepted view as to the formation of starch around the pyrenoid, as given by OLTMANNS, is that of SCHMITZ. "The origin of the starch layer out of the individual grains is very difficult to follow. If algae are taken from which the starch has been removed and put under favorable conditions for observation, there will at first appear, according to SCHMITZ, little round grains that are isolated from one another but which later grow and flatten themselves through pressure on each other." In contradistinction to this view is that of TIMBERLAKE (**21**), who found segments of the pyrenoid of Hydrodictyon breaking off and becoming converted into starch grains by the deposition of starch in each segment so cut off.

TIMBERLAKE has described many of the pyrenoids of Hydrodictyon, especially where starch formation was going on rapidly, as having two or three concentric layers of starch around them. In Cladophora (**22**) he has described the pyrenoid as a double convex lens-shaped body, from which starch grains are cut off first on one side and then on the other. These grains not being removed at once may form several layers of starch on either side. All the other pyrenoids so far described, such as those of Spirogyra, Zygnema, etc., have only a single layer of starch around them.

BÜTSCHLI (**2**) has recently made a careful study of the paramylon bodies of Euglena, and has found that in preserved material they break up into a number of disks which he regards as due to their internal structure.

The staining reactions of the pyrenoids are quite interesting and have been described by TIMBERLAKE (**21**). The pyrenoid stains a brilliant red in the triple stain, while the starch around it, and in the stroma, is colored as bright a blue.

NĚMEC (**16**) has called attention recently again to the reaction of starch to gentian violet, but uses a more complicated technique to obtain the same result that may be had with the ordinary triple stain when properly used. He soaks his sections on the slide in a 2 per cent. aqueous solution of tannin for 10–60 minutes, washes for a minute in water, puts them into a 1.5 per cent. solution of tartar emetic for 5–15 minutes, and then stains them, after washing in water, for about 30 minutes in an aqueous solution of gentian violet. By this procedure he gets what he calls an inverse staining, in which the starch is stained a brilliant violet while the cytoplasm and chromatin remain unstained. In order to stain the chromatin he suggests a nuclear stain previously with paracarmin *in toto*. This lengthy and troublesome process is entirely unnecessary however, for the triple stain used after Flemming's fixing solutions gives sharply defined starch grains stained a brilliant blue, which is permanent for years, as our slides in use in the laboratory show, while the chromatin is a red or violet. This reaction of starch in the triple stain has been noted not only by TIMBERLAKE for pyrenoid starch, but also more recently by DENNISTON (**6**) of this laboratory for the starch of the higher plants.

Methods

While Closterium is one of the commonest of the fresh water algae in this region, it does not frequently occur so abundantly that a cytological study by means of sections can be made of it. For the past three years, however, the bottoms and sides of the lily banks in the university greenhouses, as well as the larger filamentous algae, such as Oedogonium, growing on the bottom of the tanks, have been covered during the summer months by an abundant and almost pure growth of *C. Ehrenbergii* Menegh., and during the last spring with a mixed growth of *C. Ehrenbergii* and *C. moniliferum* Ehrenb.

The Closterium was so abundant in these tanks that all that was necessary was to shake some of the plants to which they were attached in a vial of water. The Closterium would sink to the bottom and form there a layer of considerable thickness. The water could then be pipetted or decanted off and the fixing fluid added. Washing the plants and changing the alcohols and paraffins were all done in the same way, either by decantation or by pipetting off the liquid above them. About twelve hours after the change to 52° paraffin the vial was set in ice water to harden the paraffin. The vial was then broken, the glass carefully picked away from the paraffin, and the bottom layer sectioned. I am indebted for this method to Dr. OVERTON of this laboratory, who has used it very successfully in sectioning Paramoecium.

Material was fixed in Flemming's medium, Flemming's weaker, and Flemming's weaker half strength, and Merkel's solutions. Flemming's weaker half solution strength gave the best results both on the nuclei and on the pyrenoids, causing very little shrinkage and preserving the internal structures well. With the exception of the pyrenoids, Merkel's solution preserved the cytoplasm structures better, but in the former caused shrinkage, as did the Flemming's medium solution. The sections were stained with the triple stain, the pyrenoid itself taking on a brilliant red color, while the starch around it always took the gentian stain as has already been described.

The chromatophore

As noted above, the only figures of the cross section of a chromatophore of Closterium, so far published, are those of NÄGELI, in which it is shown as composed of a central cylinder from which radiate ridges like the spokes of a wheel. In his figures NÄGELI does not show these ridges as extending to the cell wall, but in his description he says they do. The form of the chromatophore is quite different in *C. Ehrenbergii* from that described by NÄGELI for *C. parvulum* and *C. moniliferum.* In this form the central cylinder is quite large, occupying practically the entire cell space, while the ridges are comparatively narrow and extend practically to the cell wall, only an extremely thin layer of cytoplasm separating the latter from it. The chromatophore of *C. Ehrenbergii* in cross section (*fig. 2*), instead of resembling a hub with radiating spokes, as in NÄGELI's two forms, is more like a coarsely cogged wheel. The cytoplasm of the grooves between the ridges is sometimes very densely stained, being much darker than the chromatophore itself, but containing a great number of vacuoles of varying sizes (*figs. 1, 2*). At other times, however, these spaces between the ridges are almost free from stainable materials, showing only a few large vacuoles or having a very reticulate structure (*fig. 2*). When the green plants in the living condition are examined under the microscope, it can be noticed at once that there is great variation among them; some of them are so dark green as to be almost opaque, while others are much lighter green and are semi-transparent. It is clear that the density of the cytoplasm between the ridges determines in a large degree the external appearance of the plant. DeBary has described the chromatophore as being homogeneous, except for the pyrenoids, in surface view, but a careful examination shows that the larger vacuoles of the cytoplasm between the ridges appear as lighter areas in the darker green of the chromatophore.

The material of the chromatophore itself is in the form of a very fine reticulum, the meshes of which run out into the ridges in such a manner that in cross section the latter appear to be almost radially striate. The meshes are quite small in the outer layer of the chromatophore, but its central region shows a much coarser reticulum. The meshes here vary much in coarseness, and their structure, in connection with the cytoplasm in the grooves, determines whether the plant has a dark or light green color.

The number of ridges on the chromatophore varies from 12 to 18; this is more than is given in the systematic descriptions of this form, but it is practically impossible to count the number of ridges accurately except in sections.

Toward the ends of the Closterium the ridges seem to withdraw from the walls, and a cross section at these points shows a chromatophore with a somewhat star-shaped form and a rather thick layer of cytoplasm between the outer edges of the ridges and the cell wall (*fig. 3*). The ridges are fewer in number also, owing to the fact that some of them do not extend to the ends, and their outer edges are acute instead of blunt and rounded as is the case nearer the center.

In *C. moniliferum* (*figs. 5–7*) the structure of the chromatophore is practically the same except that the number of the ridges in my form is always 10; Nägeli's figures show 10 and 6. This gives an easy additional means of identifying the species in section, as the ridges of *C. Ehrenbergii* are always more numerous. The central core of the chromatophore is always larger than Nägeli figured it. In optical cross section it gives the appearance he shows, but actual cross sections show clearly that it is essentially similar to that of *C.*

Ehrenbergii. The relative thickness of the central core as compared with the width of the ridges is shown in *fig. 4*.

The pyrenoids of *C. Ehrenbergii* are imbedded in the substance of the chromatophore close to its surface. It is very rare, except near the ends of the plant, to find a pyrenoid placed far toward the interior of the plant. In cross section it will be seen that many are either placed in the ridges of the chromatophore or lie at their bases. Although many of them lie in the ridges, none of them are found in their extreme outer edges (*fig. 2*).

Where strands of cytoplasm run across the central part of the cell body, they seem to tend to be oriented on the pyrenoids. If streaming of the cytoplasm occurs in these strands, as so frequently happens in the algae, this arrangement would put the pyrenoid in quick communication with all parts of the cell and would facilitate the movement of food products toward or away from it. It would seem to occupy a favorable position in its location so close to the surface of the chromatophore and also in its relation to other parts of the cell.

In *C. moniliferum* there is a single row of pyrenoids down the central axis. In cross section (*figs. 6, 7*) the pyrenoids occupy the center of the chromatophore. As this species is so much smaller than *C. Ehrenbergii*, there is apparently not so much necessity for the pyrenoids being at the surface.

As mentioned above, the pyrenoids in *C. Ehrenbergii* do not lie in the ridges in all cases, although many of them do. In addition to the layer of starch which incloses each pyrenoid, there are, as in Hydrodictyon, numerous starch grains lying free in the cytoplasm. These starch grains practically all lie arranged in longitudinal series in the ridges (*fig. 1*). In a longitudinal section it is rather common to see a number of pyrenoids lying in a ridge in a row, and scattered among them a number of starch grains. The grains seem to have a tendency to collect in these parts. These free grains undoubtedly had their origin around a pyrenoid, as their shape is exactly that of such grains, being angular at the edges and concave (*fig. 8*). Whether these grains became free by a second layer of starch forming around the pyrenoid and crowding out the older layer, or by the disintegration of some of the pyrenoids themselves, or in some other way, I cannot say, but as to the common origin of both plasma starch and pyrenoid starch around some pyrenoid I can entirely confirm TIMBERLAKE'S results on Hydrodictyon. In *C. moniliferum* the stroma starch is also present and is located in the same position, at the surface of the chromatophore.

The pyrenoids

The pyrenoids of Closterium, like those of all the Conjugatae and unlike those of Hydrodictyon, as described by TIMBERLAKE, have a single layer of starch grains around them. In size the pyrenoids vary all the way from bodies almost impossible to see even with the highest magnification to spheres whose diameter is a fifteenth that of the Closterium body itself. Even the small pyrenoids have usually a little starch around them; frequently this is only on one side, but at other times it may extend all the way around (*figs. 18, 21*). These forms in which the starch occurs largely on one side are rather difficult of explanation; the one-sidedness may be due to a difference in the amount of carbohydrate food supply furnished to the two sides, or it may be due to the method of origin of the pyrenoid, as will be discussed later. A more typical form and one more frequently figured is that in which the starch lies as a mass of about uniform thickness in the form of a hollow sphere about the pyrenoid body (*figs. 10–13*). This sphere is only one layer of grains in thickness, although there were a few cases where more than one layer was suggested by the appearance of other grains just outside the regular layer. The clefts between the grains usually extend out radially, but may at times be almost tangential. The grains around any one pyrenoid vary greatly in thickness, and there are usually one or two grains of relatively larger size. The number of grains in the series around a pyrenoid is correlated with their thickness; the thicker the grains the more numerous they are and the more irregular their shape. No two pyrenoids are alike as to the shape of the grains around them. No stratification is visible either in grains or pyrenoids.

It must not be inferred that there is a correlation between the size of the pyrenoid and the thickness of the grains around it. In a general way large pyrenoids have thick grains around them (*fig. 12*), but many very small pyrenoids have thick layers of starch around them, and some of the very largest may be without starch or have only a very thin layer. It is apparent that it is the physiological condition of the plant concerned and not the size of the pyrenoids that determines this difference. This is in harmony with the observed fact that in any one Closterium the starch layers around its pyrenoids will all tend to have the same thickness.

In fixed material, at least, the starch grains are not in direct contact with the pyrenoid. Between the body of the pyrenoid itself and the starch around it there is a layer of substance which either does not stain or stains the same color as the cytoplasm. It seems natural, of course, that there must continue to be some organic connection between the pyrenoid and the starch grains surrounding it; otherwise the starch would be moved away from its pyrenoid by the streaming of the cytoplasm, which undoubtedly occurs here in the chromatophore even if not so rapidly as it does in the cytoplasm next the cell wall. Whatever the connection may be, it must be maintained through this unstained layer, which seems from its staining reaction to be neither pyrenoid nor starch.

There is great variation in the appearance of the pyrenoid itself in different specimens. In one specimen the pyrenoids may all appear angular, while in the specimen lying next to it they are full and globular. This difference may be due to differences in the way in which the fixing solutions happen to affect the particular Closterium containing the pyrenoids, but I am inclined to think that it is rather due to some condition in the pyrenoid itself. The angular form was especially noticeable where the pyrenoids were very large and with little starch. The fixation of the general cell structure with the Flemming weak, half strength, was good in these cases, and many details in the structure of the pyrenoid could be made out. It is an interesting fact that a single cell presents in general the same type of pyrenoid throughout its whole extent.

Very few of the pyrenoids are homogeneous, although they are usually described as being so; practically all of them show parts that are denser. One of the more common forms which this dense region takes is that of an irregular star-shaped portion toward the center of the pyrenoid (*fig. 15*). In this case the central part may be very irregular, with rays extending out from it. The denser portion has sometimes the appearance of a zone or band of darker staining material extending throughout the pyrenoid body (*fig. 22*). The ends of this zone may be split so as to give a Y-shaped figure in cross section. In a Closterium that had been fixed at night I have observed a large vacuole in the pyrenoid (*fig. 23*).

In a great number of cases the denser portions are numerous and are scattered apparently irregularly throughout the pyrenoid. A careful examination will show, however, that each dense area is opposite a starch grain, while the lighter portions are opposite the clefts between the grains, appearing merely as continuations of the latter (*figs. 10, 11*). This concentration of pyrenoid material so near to the place where starch is being laid down in the starch grain is an interesting fact, indicating that even though the rudiment of the grain may be formed as TIMBERLAKE has suggested some kind of organic connection is still retained between the young grain and the part of the pyrenoid nearest to it. The same relation might be expected to exist also if the grain is wholly a deposition product of the pyrenoid. How long this connection is retained cannot be determined. In any case, this increased concentration of the pyrenoid material in certain definite regions would seem to indicate that the pyrenoid is not a mere mass, but that it has a structure correlated more or less wholly with its function.

In many cases the pyrenoids seem to be in the process of cutting off pieces or of breaking up into a number of pieces. These may be in the form of a segment split off from one side (*figs. 16, 17*), or the entire pyrenoid may break up into lens-shaped segments (*figs. 25, 26*). Where the body of the pyrenoid itself shows a more densely staining band in cross section, these segments are cut off from one or both sides of this region (*fig. 16*). If the pyrenoid is made up of denser bodies placed opposite starch grains, the cutting off may occur along any of the lighter, less dense lines (*fig. 10*); however, hardly ever more than two of these segments are cut off and usually only one of them at any one time. The process may be traced in all its stages, from a lightly stained zone in the pyrenoid body to a small cleft, and finally to the separation of a free segment. TIMBERLAKE was able to find in the pyrenoids of Hydrodictyon that the segments changed their staining reactions from the bright red of the pyrenoid by a series of transition stages to the violet color characteristic of the starch, but no such series of transition stages could be found in Closterium, although the segments cut off frequently appear as if stained a lighter red, owing to their smaller size. It is a question as to what becomes of these pieces of pyrenoids. Some figures seem to suggest that they round up and form smaller pyrenoids. It is very difficult to get any series of stages showing the migration of the parts away from each other, if such is the case. The hypothesis that they form other pyrenoids is supported by the fact that the starch layer is frequently of varying thickness, and may be quite thin on one side, as would happen if a segment had been cut off that side and the layer of starch had not been redeposited to any great degree (*fig. 9*).

The great variation in size of the pyrenoids is a conspicuous fact. SCHMITZ, who first observed pyrenoid division, says distinctly that they may divide equally or very unequally. It is certainly true that they are capable of splitting up into segments, whether these segments become independent pyrenoids or develop into starch grains.

In this connection the observation of BÜTSCHLI that the paramylon bodies of Euglena split up into similar shaped disks is very interesting. He regards it as due to their internal structure. The similarity of his figures to those of the pyrenoids in Closterium may indicate that paramylon bodies and pyrenoids are alike in their essential structure, even though not in chemical composition.

It should also be stated that these clefts in the body of the pyrenoid appear also in fixed and stained specimens that have not been sectioned but are examined whole.

I have observed many of the typical figures of the division of one pyrenoid to form two in material fixed at night. These figures are in all essentials like those observed by SCHMITZ in Hyalotheca and by a number of investigators since his day. It seems to consist simply in a pinching in two of the pyrenoid, and it is a curious fact that some of these same pyrenoids show the composite structure just described (*fig. 26*).

In Hydrodictyon, according to TIMBERLAKE, the pyrenoids show no such numerous clefts as are present in Closterium, and this fact makes it still more difficult to believe that the segments in Closterium are destined to form starch grains. The origin of the starch grains and the nature and fate of the pyrenoid segments are difficult questions. That the two problems are closely related, as maintained by TIMBERLAKE, is the most suggestive conception that has so far been advanced, but the whole matter is less clear in Closterium than in Hydrodictyon. Further comparative studies on the pyrenoids of the Conjugatae will doubtless make the significance of the pyrenoid segments more clear.

A culture of Closterium was kept in the dark for three days, and then when treated with iodin showed very little starch present around the pyrenoids. Specimens from this culture were imbedded and sectioned. It was found that in most cases the starch had entirely disappeared, but that in others there were still traces of it present in the form of a thin layer around the pyrenoids (*fig. 24*). Some stroma starch was also present. The stroma starch seems to persist longer than that formed more recently around the pyrenoids. Staining the pyrenoids after keeping them in the dark was rather difficult, requiring 10–20 minutes, where before it had not taken that number of seconds; the stain was not brilliant even then. It was noticeable too that the pyrenoids had diminished one-third to one-fourth in size, showing that some of the pyrenoid substance had been used up.

The nucleus

Only a brief description of the nucleus will be given here, my principal aim at this time being to correct the erroneous view as to the shape of the chromatophore of Closterium, and to give further details as to the structure of the pyrenoids. The nucleus has in longitudinal section the form of a double convex lens (*figs. 1, 29*). Its body in both species is made up of a finely reticulate network of rather lightly staining fibers, forming meshes of almost uniform size. There seem to be no differentially stained chromatin granules on the strands. There are darker masses in the fibers which may be the net knots of the older authors; the extreme fineness of the network makes this point especially difficult to determine. The most striking part of the nucleus is the great mass of granules which lie in a group in the center of this reticulum and which stain a brilliant red in the triple stain. These bodies are globular or sometimes somewhat angular granules of varying size, apparently unconnected and lying free in the reticulum. The strands from the fine-meshed reticulum surrounding them seem to come up to these granules and apparently hold them in position. These granules in *C. Ehrenbergii* are usually arranged in a fairly compact mass of varying size at the center of the nucleus (*fig. 1*), but they may be scattered out in an irregular manner across the long axis of the nucleus (*fig. 29*). In *C. moniliferum* (*fig. 28*) these bodies form a mass that is practically spherical, but shows a few irregularities on its surface. The nucleus in this species resembles that of Spirogyra very much, but it can be made out that the central spherical mass is made up of smaller pieces, while in Spirogyra the so-called nucleole appears as a homogeneous sphere.

The real significance of these granules and of the meshwork that surrounds them, and the part which each takes in cell division, can be determined only by seeing them during the time of chromosome formation. Their behavior will throw light on the nature of the so-called chromatin nucleolus of Spirogyra, in which, as described by MITZKEWITSCH (14), BERGH (1), and others, is contained all the chromatin in the nucleus. I shall discuss the phenomena of nuclear and cell division in a further paper.

My indebtedness to Professor R. A. HARPER for his advice and criticism during the progress of this work is very great, and I take this opportunity of expressing my appreciation of them.

Summary

1. The current figures and descriptions of the chromatophore of Closterium derived from NÄGELI are fundamentally incorrect. The chromatophore is not made up of a series of radiating plates about a slender central core, but is a curved cone-shaped structure with relatively narrow ridges on its surface.

2. The pyrenoids are imbedded in the periphery of this chromatophore in *C. Ehrenbergii* and exactly at its center in *C. moniliferum*.

3. Pyrenoid starch and stroma starch both have the same origin, all the starch being formed around pyrenoids.

4. The pyrenoids show considerable internal structure, frequently containing denser and lighter portions, vacuoles, etc., and often are cleft into a mass of disks or segments of varying number and form.

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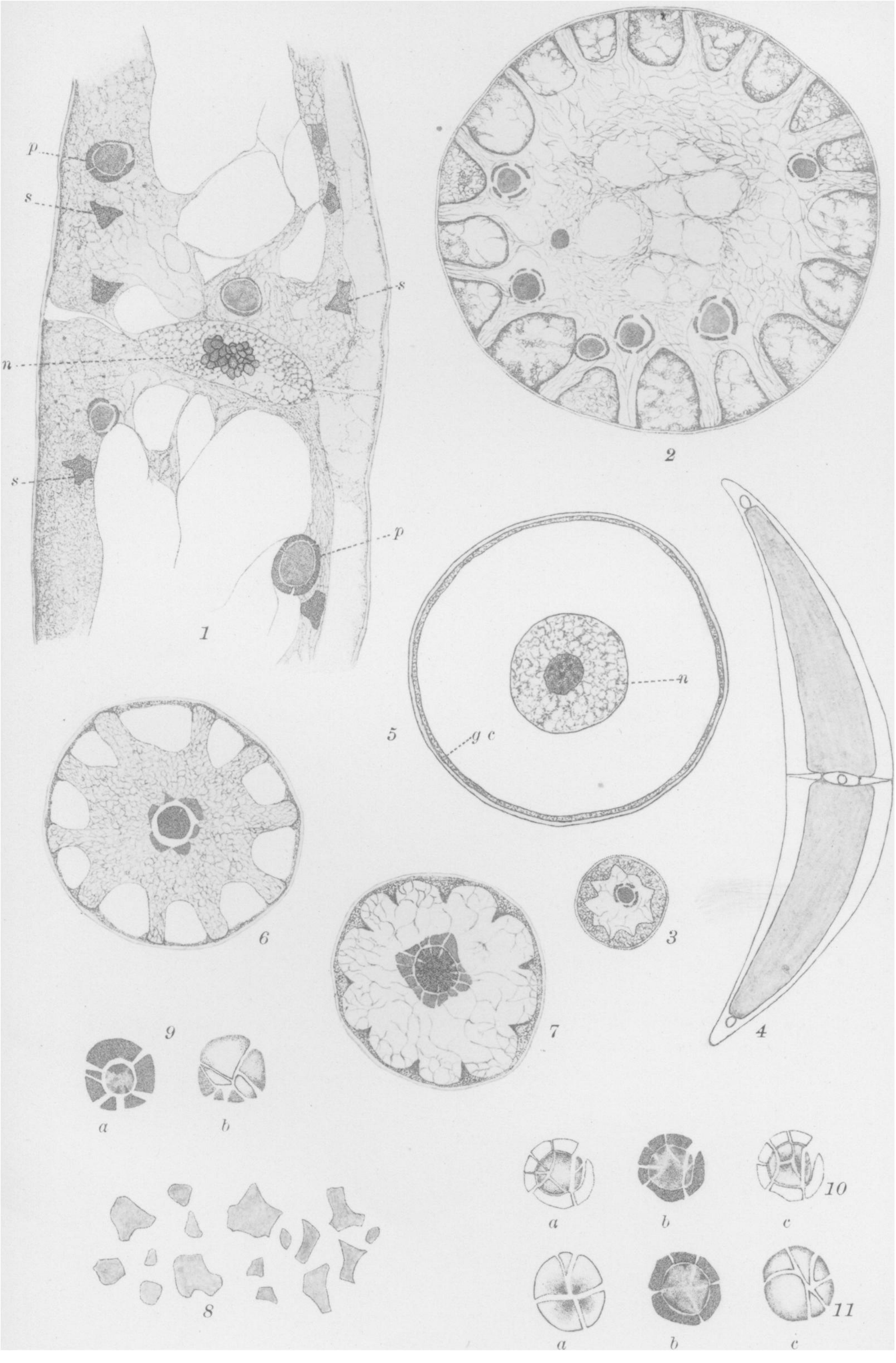

LUTMAN *on* CLOSTERIUM

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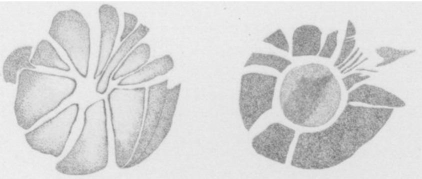

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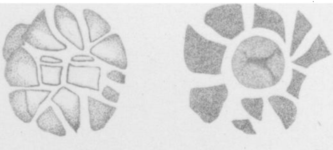

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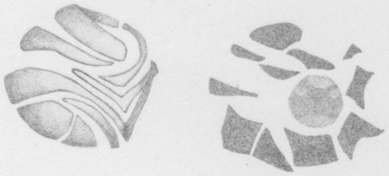

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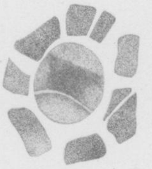

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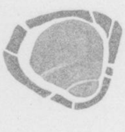

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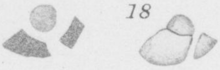

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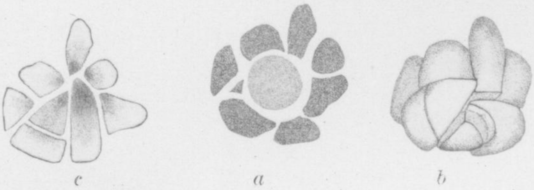

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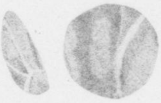

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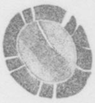

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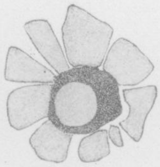

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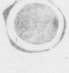

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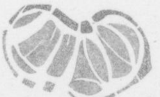

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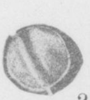

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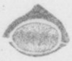

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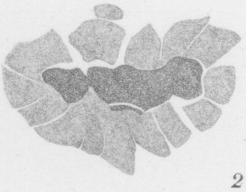

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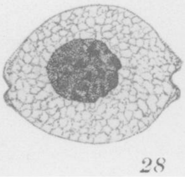

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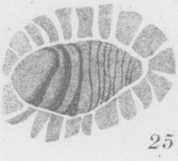

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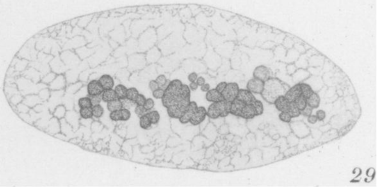

29

LUTMAN on CLOSTERIUM

HELIOTYPE CO., BOSTON.

EXPLANATION OF PLATES XVII AND XVIII

MAGNIFICATION.—*Figs. 1, 2, 5, 6, 7*, about ×900; all the others, about ×1250.

FIG. 1.—*C. Ehrenbergii;* longitudinal section; on the left the section is through one of the ridges; on the right it is through one of the furrows; *n*, nucleus; *p*, pyrenoids; *s*, stroma starch.

FIG. 2.—*C. Ehrenbergii;* cross section.

FIG. 3.—*C. Ehrenbergii;* cross section near one end, showing star-shaped chromatophore.

FIG. 4.—*C. moniliferum;* shaded portion is the central part of the chromatophore.

FIG. 5.—*C. moniliferum;* schematic cross section through the nucleus (*n*); *gc*, granular cytoplasm.

FIG. 6.—*C. moniliferum;* cross section about half-way to the tip.

FIG. 7.—*C. moniliferum;* cross section nearer the tip.

FIGS. 8–26.—*C. Ehrenbergii;* details of starch and pyrenoids.

FIG. 8.—Stroma starch.

FIG. 9.—Pyrenoid showing internal markings; *a*, in section; *b*, surface view of the starch mass.

FIG. 10.—Pyrenoid showing internal markings; *a*, section of starch showing top of pyrenoid; *b*, section of starch and pyrenoid; *c*, section showing lower part of the starch.

FIG. 11.—Pyrenoid; *a*, section; *b*, top surface of starch mass; *c*, bottom of same.

FIGS. 12, 13.—Pyrenoids; *a*, surface view of starch; *b*, section of pyrenoid.

FIG. 14.—Pyrenoid; *a*, section; *b*, surface view of starch; *c*, view of the starch at deeper level.

FIG. 15.—Pyrenoid; *a*, surface of starch; *b*, section of pyrenoid.

FIGS. 16–22.—Pyrenoids showing pieces cut off.

FIG. 23.—Pyrenoid with large vacuole.

FIG. 24.—Pyrenoid after Closterium has been kept in the darkness for three days.

FIGS. 25, 26.—Pyrenoids showing peculiar disks into which the pyrenoid body has broken up.

FIG. 27.—Pyrenoid as ordinarily figured in division.

FIG. 28.—Nucleus of *C. moniliferum.*

FIG. 29.—Nucleus of *C. Ehrenbergii.*